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New isoflavones from *Ceiba pentandra*

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Abstract

Two new isoflavones, pentandrin (**1**) and pentandrin glucoside (**2**), were isolated from the stem barks of *Ceiba pentandra* along with β-sitosterol and its 3-*O*-β-D-glucopyranoside, which was isolated for the first time from this plant. The structures of these compounds were elucidated with the help of spectroscopic techniques, while the structure of **1** was unambiguously confirmed by single-crystal X-ray diffraction studies.

Keywords: Isoflavones; *Ceiba pentandra*; Pentandrin; Pentandrin glucoside; X-ray diffraction

1. Introduction

Ceiba pentandra L. Gaertn. is a tree upto 50 m high which occurs in areas ranging from tropical America to Asia through Africa (Aubreville and Leroy, 1975). The plant is well reputed in the African traditional medicine for the treatment of many illnesses, such as, headache, dizziness, constipation, mental troubles and fever. It is also used as diuretic (Busson et al., 1965; Bouquet and Debray, 1974; Irvine, 1961). Previous work on *C. pentandra* described the isolation of a number of sesquiterpenoids (Rao et al., 1993). In our systematic search for active metabolites from Cameroonian medicinal plants, we have investigated the stem bark extract of this plant and isolated two new isoflavones: pentandrin (**1**) and pentadrin 5′-*O*-β-D-glucoside (**2**), the structures of which were elucidated with the help of single crystal X-ray diffraction technique as well as NMR spectroscopy.

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2. Results and discussion

Compound **1**, $C_{18}H_{16}O_7$, was isolated from the ethyl acetate extract of the stem bark of *C. pentandra*, as yellow solid crystals. The UV spectrum of **1** showed absorptions at 325, 262, 236, 203 nm indicating the presence of substituted aromatic rings and α, β unsaturated ketone in the molecule (Agrawal, 1989). The IR spectrum showed absorptions at 3404 (OH), 1658 (CO) and 1461 (C=C) cm^{-1}.

The ^{1}H-NMR spectra [$CDCl_3$, 400 MHz] of **1** (Table 1) showed signals characteristic of the isoflavone moieties. Two 1H doublets at δ 6.39 and 6.37 ($J_{6,8} = 2.3$ Hz) represented the aromatic H-8 and H-6 *meta* to each other. Two 1H doublets at δ 6.72 and 6.68 ($J_{2',6'} = 1.8$ Hz) were assigned to *meta* coupled aromatic H-6′ and H-2′. A 1H singlet at δ 7.86 was assigned to H-2. Another 1H singlet appeared at δ 12.8 was due to the hydrogen bonded C-5 hydroxyl proton. Three 3H singlets at δ 3.91, 3.89 and 3.86 indicated the presence of three methoxyl groups.

The ^{13}C-NMR spectra [$CDCl_3$, 100 MHz, BB, DEPT, (Table 1)] of **1** showed resonances for all 18 carbons with three methoxy, five methine and 10 quaternary carbons (Murthy and Rao, 1986). The oxygen-

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Table 1
^1H - and ^{13}C-NMR for Compounds **1** ($CDCl_3$) and **2** (DMSO)

Carbon number	**1**		**2**	
	$^1H(\delta)$ *J* (Hz)	$^{13}C(\delta)$	$^1H(\delta)$ *J* (Hz)	$^{13}C(\delta)$
2	7.86 (*s*)	153.2	8.43 (*s*)	155.1
3	–	123.7	–	122.3
4	–	180.6	–	180.0
5	–	162.7	–	161.7
6	6.37, (*d*, 2.3)	98.3	6.95, (*d*, 2.3)	98.3
7	–	165.6	–	165.3
8	6.39, (*d*, 2.3)	92.5	6.98, (*d*, 2.3)	92.4
9	–	157.8	–	157.4
10	–	106.2	–	105.3
1′	–	126.6	–	125.8
2′	6.68, (*d*, 1.8)	108.6	6.60, (*d*, 1.8)	110.1
3′	–	152.3	–	152.7
4′	–	135.8	–	138.5
5′	–	149.4	–	150.6
6′	6.72, (*d*, 1.8)	105.7	6.37, (*d*, 1.8)	107.7
1″	–	–	4.91, (*d*, 7.5)	101.0
2″	–	–	–	77.1
3″	–	–	–	76.7
4″	–	–	–	73.4
5″	–	–	–	69.9
6″	–	–	–	60.8
OCH_3	3.86 (*s*)	55.8	3.74 (*s*)	56.0
OCH_3	3.89 (*s*)	56.1	3.79 (*s*)	56.1
OCH_3	3.91 (*s*)	61.0	3.84 (*s*)	60.3

bearing carbons resonated at δ 165.6 (C-7), 162.7 (C-5), 157.8 (C-9), 153.2 (C-2), 152.3 (C-3′), 149.4 (C-5′) and 135.8 (C-4′). The carbonyl carbon and the three methoxy carbons appeared at δ 180.6, 61.0, 56.1 and 55.8, respectively.

Direct one-bond $^1H/^{13}C$ connectivities of **1** were established by HMQC (hetronuclear multiple quantum coherence) spectrum which showed that H-2 (δ 7.86) was connected with C-2 (δ 153.2). Similarly, H-6 (δ 6.37) and H-8 (δ 6.39) showed connectivity with C-6 (δ 98.3) and C-8 (δ 92.5), respectively. H-6′ (δ 6.72) and H-2′ (δ 6.68) showed HMQC connectivity with δ 105.7 (C-6′) and 108.6 (C-2′), respectively. The long-range hetronuclear interactions observed in HMBC spectrum (heteronuclear multiple bond connectivity) for **1** are shown in Fig. 1.

The HREI MS of **1** showed the M^+ at *m/z* 344.0845 corresponding to the molecular formula $C_{18}H_{16}O_7$ (calcd. 344.0847) with 11° of unsaturation in the molecule, eight of which were accounted for by the two benzene rings, one by the pyrane ring, one by the carbonyl group and one by the double bond in the pyrane ring. The fragment at *m/z* 167.0294 $[C_8H_6O_4+ H]^+$ resulted from the retro Diels Alder cleavage indicated

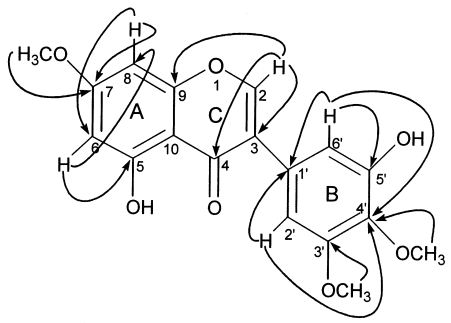

Fig. 1. Important HMBC interactions in Compound **1**.

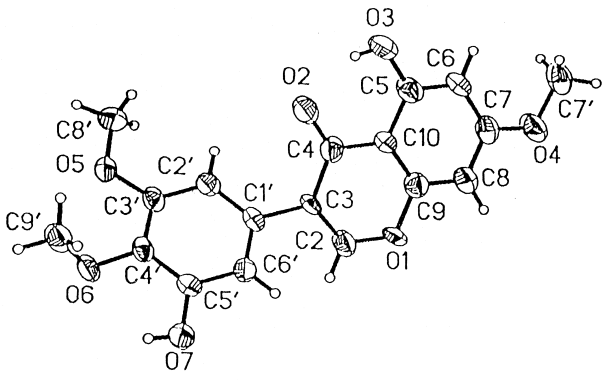

Fig. 2. A computer generated ORTEP drawing of **1**. The hydrogens shown are at arbitrary positions and refined isotropically.

the presence of one hydroxyl and one methoxy substituent in ring A (Chibber and Sharma, 1979).

On the basis of above spectral data, the compound **1** was deduced as 5,5′-dihydroxy- 7,3′,4′-trimethoxy isoflavone. Subsequent single-crystal X-ray diffraction analysis (Fig. 2) confirmed structure **1** for this compound. The molecule showed planarity throughout the structure. The average C–C bond distance in the aromatic rings is 1.386 (2) Å.

Compound (**2**), $C_{24}H_{26}O_{12}$, was isolated as a yellowish amorphous solid. The UV spectrum of **2** showed absorptions at 325, 262, 237 and 204 nm (Agrawal, 1989). The IR spectrum showed absorptions at 3406 (OH), 1657 (CO) and 1463 (C=C) cm^{-1}.

The ^{1}H-NMR [DMSO, 300 MHz, (Table 1)] spectrum of compound **2** was distinctly similar to **1**, with additional signals of a sugar moiety. The anomeric signal appeared as a doublet at δ 4.91 ($J_{1''}$, $_{2''} = 7.5$ Hz), while the other methine protons of the sugar moiety appeared between δ 3.19–5.35.

The ^{13}C-NMR spectrum of **2** also showed similarity with that of **1** except for the addition of six carbon signals of a sugar moiety which appeared at δ 101.0, 77.1, 76.7, 73.4, 69.9 and 60.8, assigned to C-1″, C-2″, C-3″, C-4″, C-5″ and C-6″, respectively. The substitution of the sugar moiety at C-5′ was inferred by the important HMBC interactions between anomeric H-1″ and C-5′. The presence of a hydrogen-bonded –OH signal at δ 12.85 also indicated that there was only C-5′ position available for the attachment of sugar moiety. The HMBC interactions are shown in Fig. 3.

The HREI MS of compound **2** showed the M^+ at m/z 506.1472 corresponding to the molecular formula $C_{24}H_{26}O_{12}$ (calcd. 506.1424) with 12° of unsaturation. The M^+ was further confirmed by FAB MS $[M + 1]^+$ at m/z 507.1472. The EI MS showed the highest peak at m/z 344.0847 $[M^+ - 162]$ indicating the presence of a sugar moiety in the molecule, which was further confirmed by ^{1}H- and ^{13}C-NMR spectra. The rest of the mass fragmentation of **2** was similar to compound **1**.

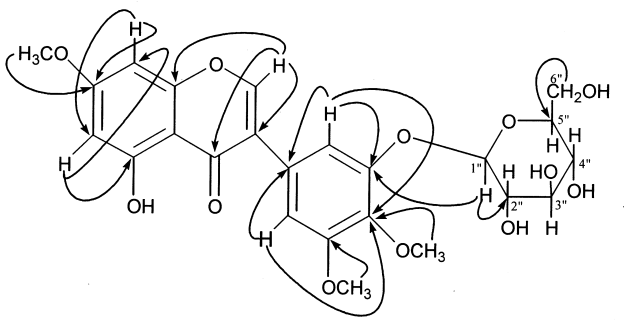

Fig. 3. Important HMBC interactions in Compound **2**.

On the basis of above spectral data, compound **2** was deduced to be the glycoside of **1**.

2.1. Acid hydrolysis of 2

Compound **2** (5 mg) was dissolved in MeOH–distilled H_2O (1:1). Then, 5% HCl (5 ml) solution was added in it and the solution was refluxed for 7 h at 60°C. After cooling, MeOH was evaporated in vacuo. The reaction mixture was extracted thrice with chloroform to remove the aglycone part of the molecule, which was identified as **1** on the basis of Co-TLC, EI MS and ^{1}H-NMR. The residue obtained after removal of the acid was compared with standard sugars on silica gel plates (E. Merck Art. No. 5554) using *n*-BuOH–EtOAc-*iso*-PrOH–HOAc–H_2O (7:20:12:7:6). The TLC was developed through multiple run. Spots were visualized with aniline phthalate reagent and the sugar was identified to be D-glucose.

3. Experimental

Vacuum liquid chromatography (*n*-hexane:ethyl acetate and ethyl acetate:methanol) was performed using silica gel (type 60, Merck). Thin layer chromatography was carried out using Merck precoated silica gel sheets (0.25 mm, 60 F_{254}). Column chromatography was performed using silica gel (230–400 mesh). Ceric sulphate spray reagent and UV light (366 nm) were used for the detection of the compounds. UV spectra were recorded on a Hitachi UV 3200 spectrophotometer. IR spectra were recorded on a JASCO 302-A spectrophotometers. Melting points were measured on a Gallenkamp melting point apparatus and were uncorrected. FAB MS measurements were carried out on a JEOL-HX 110 mass spectrometer. EI MS were recorded on a Varian MAT 311A mass spectrometer. The ^{1}H-NMR spectra were recorded on Bruker AM 400 and 300 MHz NMR spectrometers, while ^{13}C-NMR spectra were recorded at 100 and 75 MHz on the same instruments. Single crystal X-ray data were collected on Bruker P_4 diffractometer (previously Nicolet).

3.1. Crystal data and X-ray crystal structure determination of pentandrin (1)

Crystals of **1** suitable for X-ray analysis were obtained by recrystallization from methanol.

3.2. Crystal data

Light yellow prismatic crystals, dimensions 0.30 × 0.25 × 0.30 mm^3, $C_{18}H_{16}O_7$; M^+ = 344.0847 amu; Monoclinic, $\beta = 97.630$ (2)°, $a = 7.8110$ (10), $b =$

7.9510 (10), $c = 25.177$ (2) Å, $V = 1549.8$ (3) Å^3; space group $P2_1$, $Z = 4$, $D_x = 1.476$ Mg m^{-3}, $\mu = 0.970$ mm^{-1}, $F(000) = 720$; temperature = 293 (2) K.

3.3. Data collection

Bruker P_4 diffractometer (previously Nicolet), θ-2θ scan type, graphite-monochromated CuKα radiations; 3621 reflections were measured ($3.5 < \theta < 135^\circ$) of which 2877 were unique. Three standard reflections were measured after every 97 reflections, which showed no significant crystal decay. Data was corrected for Lorentz and polarization effects.

3.4. Structure analysis and refinement

The crystal structure was solved by direct methods and refined by full-matrix least squares on F^2 values [SHELXTL vr. 5.03]. Non-hydrogen atoms were refined with anisotropic temperature factors. Hydrogen atoms were included at calculated positions and refined in the riding mode. Final values of the residuals R and wR2 [for 2877 reflections with $I > 2\sigma(I)$] were 0.0469 and 0.1345, respectively. The highest and lowest peaks in final difference Fourier map were 0.274 and −0.255 eÅ^{-3}.

Table of atomic coordinates, bond lengths, bond angles and list of structure factors have been deposited at the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 IEZ, UK.

3.5. Plant material

The stem barks of *Ceiba pentandra* L. Gaertn. were collected at Mvolye Hill, Yaounde zone, Central Province of Cameroon, in May, 1998. A voucher specimen (# HNC 43623) was deposited at the National Herbarium (Yaounde, Cameroon).

4. Extraction and isolation

The stem barks of the plant was cut into small pieces, air-dried and pulverized. The resulting powder (10.3 kg) was macerated thrice at room temperature with MeOH for three days. The MeOH extracts were combined and concentrated to dryness under reduced pressure to obtain a solid (750 g), which was successively fractionated with *n*-hexane, dichloromethane and ethyl acetate. The ethyl acetate fraction showed moderate antifungal activity against *Trichophyton schoenleinii*, *T. simii* and *Microsporum canis* fungi. The ethyl acetate extract (50 g) was purified by vacuum liquid chromatography on silica gel (250 g). Elution with *n*-hexane–ethyl acetate (85:15) afforded pentandrin (**1**) (15.1 mg, 1.46×10^{-4}% yield) as yellow crystals in MeOH. Elution with *n*-hexane–ethyl acetate (1:4) furnished (**2**) (13.3 mg, 1.30×10^{-4}% yield). During this investigation, β-sitosterol and its 3-*O*-β-D-glucopyranoside were also isolated.

Pentandrin (**1**), $C_{18}H_{16}O_7$; yellow solid crystal; m.p. 159–160°; UV (MeOH) λ_{max}: 325, 262, 236, 203 nm; IR ν_{max}^{KBr} cm^{-1}: 3404 (OH), 1658 (CO), 1461 (C=C) cm^{-1}. HREI MS: m/z: 344.0845 (calcd. 344.0847) [$C_{18}H_{16}O_7$], 167.0294 [$C_8H_7O_4$, calcd. 167.0340]; ^{1}H-NMR (400 MHz, $CDCl_3$): δ Table 1, ^{13}C-NMR (100 MHz, $CDCl_3$): δ Table 1.

Pentandrin glucoside (**2**), $C_{24}H_{26}O_{12}$; yellow amorphous; m.p. 180–182°; UV (MeOH) λ_{max}: 325, 262, 237 204 nm; IR ν_{max}^{KBr} cm^{-1}: 3406 (OH), 1657 (CO), 1463 (C=C) cm^{-1}. HREI MS: m/z: 506.1472 (calcd. 506.1424) [$C_{24}H_{26}O_{12}$], 344.0845 (calcd. 344.0847) [$C_{18}H_{16}O_7$], 167.0294 [$C_8H_7O_4$, calcd. 167.0340]; ^{1}H-NMR (300 MHz, DMSO): δ Table 1, ^{13}C-NMR (75 MHz, DMSO): δ Table 1.

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